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Authors: Oksana Iamshanova
Alessandra Fiorio Pla
Natalia Prevarskaya

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Molecular mechanisms of tumor invasion: regulation by calcium signals

Oksana Iamshanova, Alessandra Fiorio Pla & Natalia Prevarskaya[#]

Inserm U1003, Laboratory of Excellence, Ion Channels Science and Therapeutics, Equipe Labellisée par la Ligue Nationale Contre le Cancer, SIRIC ONCOLille, Université des Sciences et Technologies de Lille, 59656 Villeneuve d'Ascq, France

[#] Corresponding author: Prof. Natalia Prevarskaya,
Inserm U1003, Laboratory of Excellence, Ion Channels Science and Therapeutics, Equipe Labellisée par la Ligue Nationale Contre le Cancer, SIRIC ONCOLille, Université des Sciences et Technologies de Lille, 59656 Villeneuve d'Ascq, France
Tel: +33 3 20 33 64 23
Fax: +33 3 20 43 40 66
Email: natacha.prevarskaya@univ-lille1.fr
http://www.phycell.univ-lille1.fr

Abstract

Intracellular calcium (Ca^{2+}) signals are key regulators of multiple cellular functions: both healthy and physiopathological. It is therefore unsurprising that several cancers present a strong Ca^{2+} homeostasis deregulation. Among the various hallmarks of cancer disease, a particular role is played by metastasis, which has a critical impact on cancer patients' outcome. Importantly, Ca^{2+} signalling has been reported to control multiple aspects of the adaptive metastatic cancer cell behavior, including epithelial-mesenchymal transition, cell migration, local invasion and induction of angiogenesis (Fig. 1). In this context Ca^{2+} signalling is considered to be a substantial intracellular tool that regulates the dynamicity and complexity of the metastatic cascade. In the present study we review the spatial and temporal organization of Ca^{2+} fluxes, as well as the molecular mechanisms involved in metastasis, analyzing the key steps, which regulate initial tumor spread.

Abbreviations

Arachidonic acid, AA; calcium, Ca^{2+}; cyclic AMP, cAMP; endothelial cells, ECs; endoplasmic reticulum, ER; epidermal growth factor, EGF; epithelial-mesenchymal transition, EMT; extracellular matrix, ECM; hydrogen peroxide, H_2O_2; sulfhydric acid, H_2S; human umbilical vein endothelial cell, HUVEC; Ca^{2+} activated potassium channels, KCa; matrix metalloproteinase, MMP; membrane type 1 matrix metalloproteinase, MT1-MMP ; Ca^{2+}-dependent myosin light chain kinase, MLCK; myosin II, myo II; sodium, Na^+; Na^+-Ca^{2+} exchanger, NCX; nuclear factor of activated T-cells, NFAT; nitric oxyde, NO; Ca^{2+} release-activated Ca^{2+} channel protein, ORAI; ligand gated Ca^{2+} channels, P2X; purinergic G protein-coupled receptors, P2Y; phospholipase C, PLC; plasma membrane ATPase, PMCA; proline-rich tyrosine kinase 2, Pyk2; reactive oxygen species, ROS; sarco/endoplasmic reticulum Ca^{2+}-ATPase, SERCA; store-operated Ca^{2+} entry, SOCE; stromal interaction molecule, STIM; tumor-derived endothelial cells, TECs; transforming growth factor β1, TGF-β1; two-pore channel, TPC; transient receptor potential channel, TRP channel; transient receptor potential ankyrin channel, TRPA; transient receptor potential canonical channel, TRPC; transient receptor potential melastatin channel, TRPM; transient receptor potential vanilloid channel, TRPV; vascular endothelial growth factor, VEGF; voltage-gated Ca^{2+} channels, VGCCs.

Introduction

Calcium (Ca^{2+}) is an ubiquitous second messenger which is involved in the tuning of multiple fundamental cellular functions (Berridge MJ, Lipp P, 2000). Due to its multifaceted roles, it is not therefore surprising that deregulated Ca^{2+} homeostasis has been observed in various disorders, including tumorigenesis (Monteith GR, McAndrew D, Faddy HM, 2007; Prevarskaya *et al.*, 2011). Among the various manifestations of cancer, a particular role is played by metastasis, which has a critical impact on cancer patients' outcome (Hanahan & Weinberg, 2011). Tumor spread is a highly regulated process that usually starts with the loss of cell-cell contact and the epithelial-mesenchymal transition (EMT) (Kalluri & Weinberg, 2009). During metastasis, cancer cells also acquire enhanced directional movement and activate molecular pathways that enable the proteolysis of an extracellular matrix (ECM) as well as local angiogenesis. As a result, cancer cells enter the body circulation systems and disseminate to distinct sites around the organism. Importantly, Ca^{2+} signalling has been reported to control multiple aspects of the adaptive metastatic cancer cell behaviors, including EMT, migration, local angiogenesis induction and intravasation (Chen *et al.*, 2013). In this context, it is considered to be a substantial intracellular tool that regulates the dynamicity and complexity of the metastatic cascade. Intracellular free Ca^{2+} concentration is highly controlled by the fine regulation of "ON and OFF" mechanisms that ultimately generate Ca^{2+} signals with various amplitudes and frequencies. As regarding the "ON" mechanisms, cytosolic Ca^{2+} can either be delivered from extracellular space due to the activity of Ca^{2+}-permeable channels and transporters in plasma membrane, or occur as a result of a release from Ca^{2+} -containing organelles (e.g. endoplasmic reticulum) (Berridge MJ, Lipp P, 2000). In order to maintain low resting Ca^{2+} concentration, cells remove Ca^{2+} using an energy-dependent mechanism, such as plasma membrane ATPases (PMCA), or Na^{+}-Ca^{2+} exchanger (NCX). Moreover Ca^{2+} is sequestered intracellularly into Ca^{2+} -containing organelles, primarily endoplasmic reticulum (ER), by means of mechanisms which require either ATP hydrolysis (e.g. a SERCA pump), or a favorable electrochemical gradient. In this review we will overview the spatial and temporal organization of Ca^{2+} fluxes as well as those molecular mechanisms involved in metastasis, analyzing the key steps which regulate tumor spread.

Epithelial-mesenchymal transition and loss of cell-cell contact

EMT is a cellular process during which epithelial cells acquire a fibroblast-like morphology. This process involves changes in cellular shape, a loss of epithelial polarized organization and cell-cell contacts like tight and adherens junctions. Accordingly, one of the most recognized features of cells undergoing EMT, is a suppression of multiple epithelial markers (*e.g.* E-cadherin, claudins, occludins) and an overexpression of mesenchymal markers (*e.g.* N-cadherin, vimentin, integrins) (Fig.2).

Of note, EMT and the disruption of cell-cell contact is one of the key events in tumor progression. This can be induced by various effectors like growth factors, hypoxia, and inflammation (Diepenbruck & Christofori, 2016). Interestingly, the remodeling of Ca^{2+} signals during EMT processes has been reported for a variety of cancer cells. For example, in breast cancer cells, the potency of ATP-mediated cytosolic Ca^{2+} transients exhibits significant changes after epidermal growth factor (EGF) and hypoxia-induced EMT (Davis *et al.*, 2011; Azimi *et al.*, 2016). Specifically, an attenuation of the cytosolic Ca^{2+} peak and a sustained phase of Ca^{2+} influx in the response to ATP, have been attributed to the activity of G-protein-coupled purinergic receptors (P2Y family) and ligand-gated Ca^{2+} channels (P2X family) (Davis *et al.*, 2011; Azimi *et al.*, 2016). Another study reveals that an inhibition of P2X5 reduces the expression of the EMT marker vimentin, whereas its increased expression correlates with breast cancer cells that are associated with a more mesenchymal phenotype (Davis *et al.*, 2011). Moreover, the chelation of free cytosolic Ca^{2+} suppresses the production of mesenchymal markers like vimentin, N-Cadherin and CD44, after the exposure of breast cancer cells to EGF and hypoxia (Davis *et al.*, 2013; Stewart *et al.*, 2015). Similar findings have been reported for hepatic cancer cells, where chelation of intracellular Ca^{2+} reversed doxorubicin-induced EMT (Wen *et al.*, 2016). Furthermore, the EMT of colon cancer cells may be regulated by the small conductance calcium-activated channel, subfamily N, such as KCNN4 through Ca^{2+}-dependent mechanisms (Lai *et al.*, 2013). Regarding the store-operated Ca^{2+} entry (SOCE), the data is ambiguous. On one hand, SOCE and basal Ca^{2+} influx are reduced after the EGF-induction of EMT in the MDA-MD-468 breast cancer cell line (Davis *et al.*, 2012). On the other hand, the transforming growth factor β1 (TGF-β1)-induced EMT is associated with enhanced SOCE in the MCF-7 breast cancer cell line (Hu *et al.*, 2011).

It is now clear that the remodelling of Ca^{2+} signalling is a prominent feature of EMT in various cancer types. Therefore, a deregulation of Ca^{2+}-permeable channels could subserve as an important EMT regulator during carcinogenesis. Indeed, silencing and pharmacological

inhibition of transient receptor potential melastatin channels (TRPM) such as TRPM7 and TRPM8 reduce the expression of a variety of mesenchymal markers in breast cancer cells (Davis *et al.*, 2013; Liu *et al.*, 2014). In the MCF-7 breast cancer cell line that exhibits a more epithelial-like phenotype, the overexpression of TRPM8 leads to EMT induction as indicated by the profile of markers expressed (Liu *et al.*, 2014). Consistent with this data, TRPM8 has been found to be upregulated in breast cancer tumor tissues, when compared to adjacent nontumor tissues, thereby suggesting the role of TRPM8 as a determinant of EMT transition (Liu *et al.*, 2014). Moreover, in breast cancer cells, EGF-induced EMT significantly increases the mRNA level of Ca^{2+} release-activated Ca^{2+} channel protein 1 (ORAI1) and leads to altered Ca^{2+} signalling, possibly due to the involvement of transient receptor potential canonical channel type 1 (TRPC1) (Davis *et al.*, 2012). In hepatic cancer cells, another member of the transient receptor potential canonical channel family, TRPC6 has been shown to affect the expression of EMT markers after doxorubicin induction (Wen *et al.*, 2016).

Overall, the studies of Ca^{2+} signalling and Ca^{2+}-permeable channels using various cancer models and EMT effectors have defined a critical role for the Ca^{2+} signal in the EMT process during tumorigenesis.

Cell migration

The principal component of cancer cell motility is directional migration, which is due to front-rear end polarity (Mayor & Etienne-Manneville, 2016). Typically, the leading edge is represented by flat cell membrane extensions with directed actin polymerization and nascent attachment sites, whereas at the rear of the cell, adhesions are disassembled and the trailing edge is contracted (Mayor & Etienne-Manneville, 2016). Interestingly, global cytosolic Ca^{2+} is generally higher at the rear end, whereas Ca^{2+} flickers are enriched near the front edge (Evans *et al.*, 2007; Wei *et al.*, 2009; Tsai & Meyer, 2012). It is suggested that such Ca^{2+} distribution is involved in controlling the directed cellular locomotion (Brundage RA, Fogarty KE, Tuft RA, 1991).

Of note, migration is a complex and multistep process that involves coordination between cytoskeleton remodeling, cell-substrate adhesion/detachment and cellular protrusion/contraction (Gardel *et al.*, 2010; Thomas Parsons *et al.*, 2010). Importantly, several key molecular components and signalling events of the cellular migration machinery are Ca^{2+}-sensitive (Fig. 3). For example, the myosin II-based (myo II) actomyosin contraction is mainly mediated through the activity of Ca^{2+}-dependent myosin light chain kinase (MLCK)

(Clark *et al.*, 2007). The focal adhesion turnover is also highly dependent on Ca^{2+} signalling. On the one hand, the disassembly of cell adhesions is achieved due to the cleavage of focal adhesion proteins, such as integrins, talin, vinculin and focal adhesion kinases, by the Ca^{2+}-sensitive protease, calpain (Franco SJ, 2005). On the other hand, Ca^{2+} is important for the modulation of nascent focal adhesion sites by activating proline-rich tyrosine kinase 2 (Pyk2), and small GTPases like Ras and Rac (Lysechko *et al.*, 2010; Selitrennik & Lev, 2015). S100 proteins, a subgroup of the EF-hand Ca^{2+}-binding protein family, regulate a variety of cellular processes via an interaction with different target proteins (Bresnick *et al.*, 2015). In particular, their influence on F-actin polymerization and myo II-actin assembly has been suggested as governing cell migration due to the cytoskeletal structural remodeling (Gross *et al.*, 2014) (Fig.3). Overall, it is now clear that cell migration can be considered as a Ca^{2+}-dependent process. Importantly, Ca^{2+}-permeable channels are responsible for the cytosolic Ca^{2+} delivery from external and internal cellular stores. Therefore, their activity would define the occurrence of those sustained and transient Ca^{2+} changes which are important for the orchestration of cellular migration.

Interestingly, in migrating erythrocytes and human umbilical vein endothelial cells, the low basal Ca^{2+} levels at the leading edge are maintained due to the activity of PMCA and the inhibition of PMCA leads to an abrogated front-to-rear Ca^{2+} gradient and decreased migration (Pérez-Gordones *et al.*, 2009; Tsai *et al.*, 2014). Similar mechanisms could be utilized by the metastatic cells, since the expression of PMCA has been found to directly correlate with the tumorigenicity of breast cancer cells (Lee *et al.*, 2005) (Fig. 3). At the same time, in the front end of ER, low local Ca^{2+} concentration provokes high sensitivity to SOCE (Tsai *et al.*, 2014). Indeed, the ER residual Ca^{2+} sensor of SOCE, stromal interaction molecule (STIM), has been found to be distributed along the polar axis in the leading edge of the migrating cell (Tsai *et al.*, 2014). The STIM molecule responds to the Ca^{2+} ER depletion and provokes ion influx through the plasma membrane ORAI channel (Liou *et al.*, 2005; Roos *et al.*, 2005). Of note, STIM-ORAI proteins have been found to be significantly upregulated in various cancer types and SOCE-activated Ca^{2+} signalling is implemented in the mediation of actomyosin assembly and the focal adhesions required for efficient migration (Chen *et al.*, 2011; Fiorio Pla *et al.*, 2016; Jardin I, 2016) (Fig. 3).

Plasma membrane extensions and protrusions play the role of a mechanical stress and thus provide Ca^{2+} influx through stretch-activated channels at the front end of a migrating cell. Indeed, TRPM7 can be activated intracellularly through phospholipase C (PLC), or by a membrane stretch (Su *et al.*, 2006; Wei *et al.*, 2009; Gao *et al.*, 2011; Middelbeek *et al.*,

2012). Interestingly, TRPM7 is located in close proximity to calpain and myo II (Clark *et al.*, 2007). Therefore, Ca^{2+} entry provided through TRPM7 modulates actomyosin cytoskeleton contraction, as well as the dynamics of the focal adhesion turnover required for directional cell migration (Clark *et al.*, 2007). Indeed, the pro-migratory role of TRPM7 has been demonstrated for breast, lung, pancreatic and nasopharyngeal cancers (Visser *et al.*, 2014). Moreover recently, the mechano-sensitive TRPC1 activation, located at the rear of the cells, has been shown to play a role in the formation of cell polarity of U2OS bone osteosarcoma cells and their directional migration (Huang *et al.*, 2015). Similarly, several members of TRP channel family have been implicated in cell migration in various cancer types (Fiorio Pla & Gkika, 2013). In particular most TRP channels have been associated with an increase in migration potential. This is the case for TRPC members such as TRPC1, TRPC6 in glioma cells (Chigurupati *et al.*, 2010; Bomben *et al.*, 2011). In addition, the vanilloid subfamily TRPV2, has also been associated with increased cellular migration in prostate, bladder and breast cancer (Oulidi *et al.*, 2013; Gambade *et al.*, 2016). In contrast, full length TRPM8, has been reported to inhibit cell migration, thus suggesting a protective role for TRPM8 in prostate metastatic cancer progression (Gkika *et al.*, 2010, 2015), whereas the short TRPM8 isoform could have a pro-metastatic potential (Peng *et al.*, 2015; Bidaux *et al.*, 2016).

Voltage-gated Ca^{2+} channels (VGCCs) present another pathway for Ca^{2+} influx that activates a downstream MAPK/ERK signalling pathway and increases migration (Mertens-Walker *et al.*, 2010). In particular, Cav1.3 has been found to be overexpressed in endometrial carcinoma and its knockdown has been shown to reduce migration (Hao *et al.*, 2015). Indeed, at filopodia tips increased Ca^{2+} concentration provided through L-type VGCCs directs cancer cell migration due to calpain activation (Jacquemet *et al.*, 2016).

Intracellular Ca^{2+} is an important regulator of Ca^{2+} activated potassium channels (KCa). Furthermore, ORAI and TRPC1 channels may form complexes with small conductance KCa channel SK3 (Chantome *et al.*, 2013; Guéguinou *et al.*, 2016). Such a SK3-ORAI complex is crucial for the migratory function of breast and prostate cancer cells and has been found in bone metastasis (Chantome *et al.*, 2013). Similarly, colon cancer cell migration is dependent on SOCE through the SK3/TRPC1/Orai1 channel complex (Guéguinou *et al.*, 2016).

Invasiveness and invadopodia formation

The invasiveness of cancer cells is due to their ability to degrade ECM and migrate into neighboring connective tissues as well as the lymph- and bloodstreams. There, cancer cells spread throughout the organism and give rise to secondary tumor outbursts, metastases. Consequently, the understanding and hence prevention of the process of cancer cell invasion would remarkably improve the survival rate of cancer patients. Cancer cell invasion is achieved due to special structures – invadopodia, which are dynamic actin-enriched cell protrusions with proteolytic activity. Typically, the invadopodia formation process can be differentiated into the following steps: initiation, assembly and maturation (Fig.4) (Jacob *et al.*, 2015). The assembly of invadopodia is initiated in response to the focal generation of phosphatidylinositol-3,4-biphosphate and the activation of the nonreceptor tyrosine kinase Src (Mader *et al.*, 2011; Pan *et al.*, 2011; Yamaguchi H & Sakai R, 2011). The matured invadopodia recruit proteolytic enzymes, such as membrane type 1 (MT1)–matrix metalloproteinase (MMP), MMP2, and MMP9, to facilitate the focal degradation of the extracellular matrix and allow cell invasion (Beaty *et al.*, 2013).

Intruigingly, a particular pattern of Ca^{2+} signalling, Ca^{2+} oscillations, has been revealed as predisposing factor for invadopodia formation and activity (Fig. 4) (Sun *et al.*, 2014). For example, Ca^{2+} oscillations mediated through STIM1 and ORAI1 channels have been reported to activate Src kinase and hence facilitate the assembly of invadopodial precursors in melanoma cells (Sun *et al.*, 2014). The proteolytic activity of invadopodia is predetermined by the incorporation of MMP-containing endocytic vesicles into the plasma membrane at the ECM degradation sites and can also be linked to Ca^{2+} signalling machinery (Bravo-Cordero JJ *et al.*, 2007). Indeed, the inhibition of SOCE-abrogated fusion of MMP-containing vesicles with the plasma membrane result in a constrained ECM degradation (Sun *et al.*, 2014). Moreover, constitutively active TRPV2 engenders an intracellular Ca^{2+} increase and has been associated with an upregulation of MMP9 and the invasive potential of prostate cancer cells (Monet *et al.*, 2010). In oral squamous carcinoma, TRPM8 activity directly correlates with MMP9 activity and the metastatic potential of cells (Okamoto *et al.*, 2012). The downregulation of MMP9 might also be achieved after the inhibition of VGCCs (Kato *et al.*, 2007). Furthermore, in the highly metastatic MDA-MB-435 human breast cancer cell line, the activity of the ATP-gated Ca^{2+}-permeable P2X7 receptor increases invasion by the release of gelatinolytic cysteine cathepsins (Jelassi *et al.*, 2011). Therefore, in invadopodia, Ca^{2+} influx is required for the focal degradation of ECM, in particular through the upregulation of proteolytic enzymes like MMPs and cathepsins, whereas Ca^{2+} oscillations are required for the initiation of the invadopodia formation process (Fig. 4).

Induction of local angiogenesis

The first mechanical and functional interface between blood and tissues is blood vessels. Thus, in order to sustain metastatic dissemination, as well as providing sufficient metabolic support, tumor neovascularization is required. Indeed, tumor cells enable the 'activation' of the nearby endothelial cells (ECs) due to the secretion of specific molecules, such as vascular endothelial growth factor (VEGF). Hence, the complex and multistep process of angiogenesis is achieved due to the proliferation, migration, differentiation and stabilization of such tumor-derived ECs (TECs) in a new circulatory network (Carmeliet, 2005; Folkman J, 2006).

It should be noted that in tumors of various origins, Ca^{2+} signalling has been shown to regulate the release of VEGF and hence modulate angiogenesis (Fig. 5). For example, plasma membrane Cav3.2 and ER-residing STIM1 proteins have been shown to promote angiogenesis *in vivo* due to the stimulation of VEGF secretion in both prostate and cervical cancers (Chen *et al.*, 2011; Warnier *et al.*, 2015). Moreover, the importance of TRPC6 as a modulator of angiogenic potential has been revealed in glioma cells (Chigurupati *et al.*, 2010). According to this study, the number of EC branch points decreases after growing in a conditioned medium harvested from glioma cells, where hypoxia-induced TRPC6 overexpression and NFAT activation are inhibited (Chigurupati *et al.*, 2010). Of note, methyl syringate, which is suggested to be a highly specific and selective agonist of transient receptor potential ankyrin channel 1 (TRPA1) suppresses the hypoxia-induced migration, invasion and secretion of VEGF in human lung epithelial cells (Park *et al.*, 2016).

Importantly, during tumor neovascularization, the remodeling of Ca^{2+} machinery has been associated not only with the angiogenic potential of cancer cells, but also with the distinct functions of the 'activated' endothelium cells (Fig. 5). Indeed, proangiogenic Ca^{2+} signals and their related pathways are significantly altered in TEC, compared with normal EC (Fiorio Pla & Munaron, 2014). As an example, Ca^{2+} signals mediated by specific factors like VEGF and ATP and intracellular messengers such as arachidonic acid (AA), nitric oxyde (NO), or sulfhydric acid (H_2S) and cyclic AMP (cAMP) are involved in pro-migratory effects in TEC, but not in normal EC (Fiorio Pla *et al.*, 2008, 2010, 2012*b*; Pupo *et al.*, 2011; Avanzato *et al.*, 2016).

Moreover, the role of intracellular Ca^{2+} increase has been investigated at length in endothelium (Fiorio Pla & Munaron, 2014). Both pro- and antiangiogenic molecules can induce an intracellular Ca^{2+} increase, often leading to different biological effects. For instance, Ca^{2+} entry triggered by VEGF, as well as by other proangiogenic factors, is often

associated with an increase in vessel permeability, EC survival/proliferation, migration and *in vitro* tubulogenesis (Dragoni *et al.*, 2011, 2015; Li *et al.*, 2011). These outcomes can be achieved by the activation of a distinct intracellular mechanism, such as SOCE via ORAI and TRPC1 channels (Mehta *et al.*, 2003; Paria *et al.*, 2004; Jho *et al.*, 2005; Abdullaev *et al.*, 2008; Dragoni *et al.*, 2011; Li *et al.*, 2011; Fiorio Pla & Munaron, 2014), non-SOCE mechanisms via TRPC6 channels (Cheng *et al.*, 2006; Hamdollah Zadeh *et al.*, 2008), the specific engagement of the two-pore channel TPC2 subtype on acidic intracellular Ca^{2+} stores, resulting in Ca^{2+} release and angiogenic responses (Favia *et al.*, 2014), or by reverse mode activation of NCX (Fig. 5) (Andrikopoulos *et al.*, 2011). Of note, in a recent study VEGF-mediated Ca^{2+} signalling in individual endothelial cells has been investigated and shown to correlate both stochastic and deterministic response characteristics to the selection of phenotype-associated angiogenesis. In particular, altering the amount of VEGF signalling in endothelial cells by stimulating them with different VEGF concentrations, triggered distinct and mutually exclusive dynamic Ca^{2+} signalling responses, that correlated with different cellular behaviors such as cell proliferation, (monitored by NFAT nuclear translocation) or cell migration (involving MLCK) (Noren *et al.*, 2016). The *in vivo* role of Ca^{2+} signals has been recently studied in zebrafish, during angiogenic input by means of high-speed, three-dimensional time-lapse imaging to describe intracellular Ca^{2+} dynamics in ECs at single-cell resolution (Yokota *et al.*, 2015; Noren *et al.*, 2016). It may be noted that TRP Ca^{2+}-permeable channels have profound effects on the control of different steps of tumor angiogenesis. Besides their role in the VEGF-mediated Ca^{2+}-signals previously described, several data clearly show their involvement Ca^{2+} mediated signal transduction with prominent roles in tumor angiogenesis. In this context, TRPV4 is an emerging player in angiogenesis, as on EC it acts as a mechano-sensor during changes in cell morphology, cell swelling and shear stress. TRPV4 plays a significant role in endothelial migration, (Fiorio Pla *et al.*, 2012*b*) displaying a marked increase in EC derived from human breast carcinomas, as compared with 'normal' EC, leading to a greater Ca^{2+} entry that in turn activates migration in TEC (Fig. 5) (Fiorio Pla *et al.*, 2012*b*). Moreover, TRPV4 has recently been described as an important player in tumor vasculature normalization, therefore potentially improving cancer therapies (Adapala *et al.*, 2015; Thoppil *et al.*, 2015, 2016). In addition, TRPM2 has been recently identified as mediating an H_2O_2-dependent increase in macro-vascular pulmonary EC permeability (Fig. 5) (Hecquet *et al.*, 2008; Mittal *et al.*, 2015). TRPM7 inhibits HUVEC proliferation and migration, whereas its functions in human mammary epithelial cells seem to be opposite (Fig. 5) (Inoue & Xiong, 2009; Baldoli & Maier, 2012; Baldoli *et al.*, 2013; Zeng

et al., 2015). Recently, TRPA1 has been found to have a role in the vasodilatation of cerebral arteries, via an increase in Ca^{2+} influx generated by the detection of ROS, a process that requires the peroxidation of membrane lipids (Sullivan *et al.*, 2015). Similarly, TRPV2 has been shown to be expressed in aorta endothelium, but no clear functional data have been reported (Earley, 2011).

Finally, the emerging family of mechanosensitive Piezo channels has recently been described in vascular endothelial cells: Piezo2 knockdown is involved in glioma angiogenesis, both *in vitro* as well as *in vivo* by promoting abnormal intracellular Ca^{2+}, Wnt11/β-catenin signalling reduction, leading to altered angiogenic activity of endothelial cells (Fig. 5) (Yang *et al.*, 2016).

In conclusion, due to its multifaceted role in the control of endothelium homeostasis, Ca^{2+} machinery is a potential molecular target for strategies against tumor neovascularization.

Conclusions

A remodeling of Ca^{2+} signalling plays an important role during tumorigenesis. Interestingly, there are some specific channel patterns through which such Ca^{2+} signals occur, at the different stages of cancer progression. This could be partially explained by the specificity of Ca^{2+} flux, its compartment localization and the proximity of downstream Ca^{2+}-dependent targets. Furthermore, some ion channels represent multimodal activity and are characterized as not only Ca^{2+}-permeable pore proteins, but also possess other functional domains. For example, the C-terminal end of TRPM7 is constituted by a serine/threonine protein kinase domain and hence, due to the phosphorylation of cytoskeletal components, regulates cellular migration (Clark *et al.*, 2008).

Importantly, plasma membrane Ca^{2+} channels are easily and directly accessible via the bloodstream. Therefore, they are potential targets for a variety of therapeutic strategies, such as their regulation on a transcriptional and translational level, their trafficking to the plasma membrane or their stabilization at the plasma membrane (Gkika & Prevarskaya, 2009; Fiorio Pla *et al.*, 2012*a*; Bernardini *et al.*, 2015; Earley & Brayden, 2015).

Competing interests

The authors have no conflicts of interest to declare.

Authors' contributions

Oksana Iamshanova and Prof Alessandra Fiorio Pla collected information, conceived the concept, prepared figures, and drafted the manuscript. Prof Natalia Prevarskaya was involved in supervising and editing the manuscript. All of the authors read and approved the final manuscript.

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Figure legends

Fig. 1. Abstract figure. Model of the specific patterns of Ca^{2+} signals that are associated with different steps of cancer progression. It is important to note that most of the findings on Ca^{2+} signalling have been generated *in vitro*. Therefore, it is possible that in tissues, Ca^{2+} signals do not simply follow the same patterns.

Fig. 2. Epithelial-to-mesenchymal transition (EMT) and a loss of cell-cell contacts and downregulation of proteins such as E-cadherin, Claudin or Occludin. EMT transition is accompanied by the changes in Ca^{2+} signals due to several factors such as growth factors, cytokines or hypoxia. The most studied Ca^{2+}-permeable channels, which are associated with EMT, are indicated.

Fig. 3. Global cytosolic Ca^{2+} is generally higher at the rear (marked in red), whereas Ca^{2+} flickers are enriched near the front edge of migrating cell. The key molecular components and signalling events of the cellular migration machinery are Ca^{2+}-dependent. The most studied Ca^{2+}-permeable channels, which are associated with directional migration, are indicated.

Fig. 4. Ca^{2+} oscillations are required for the initiation of the invadopodia formation process, whereas Ca^{2+} influx activates the focal degradation of the extracellular matrix (ECM), in particular through the upregulation of the proteolytic enzymes like matrix metallo-proteinases (MMPs) and cathepsins. The most studied Ca^{2+}-permeable channels, which are associated with invasiveness, are indicated.

Fig. 5. Induction of local angiogenesis by Ca^{2+} signalling remodeling. In tumor cells Ca^{2+} signals regulate the secretion of proangiogenic stimuli, like vascular endothelial growth factor (VEGF). A newly formed vessel can be differentiated into following structures: tip – represented by the migrating edge of the vessel; stalk – mostly proliferating part of the vessel; phalanx – tightly apposed, regularly ordered ECs that provide perfusion and oxygenation; mural – functional preformed ECs. Interestingly, VEGF and ATP-mediated Ca^{2+} signals provide proangiogenic effects specifically on tumor derived tissue and not on healthy ECs. The most studied Ca^{2+}-permeable channels, which are associated with local angiogenesis, are indicated.

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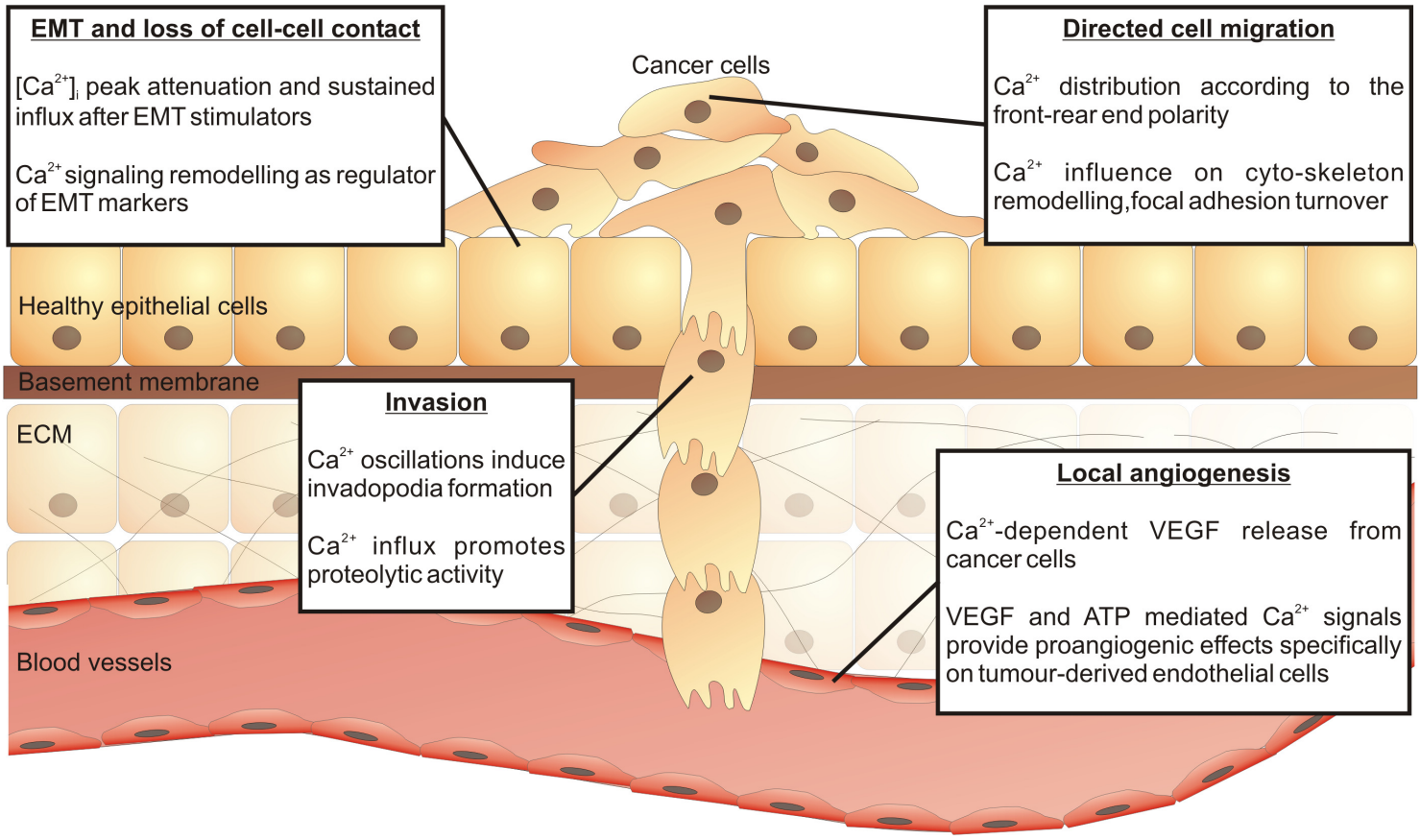
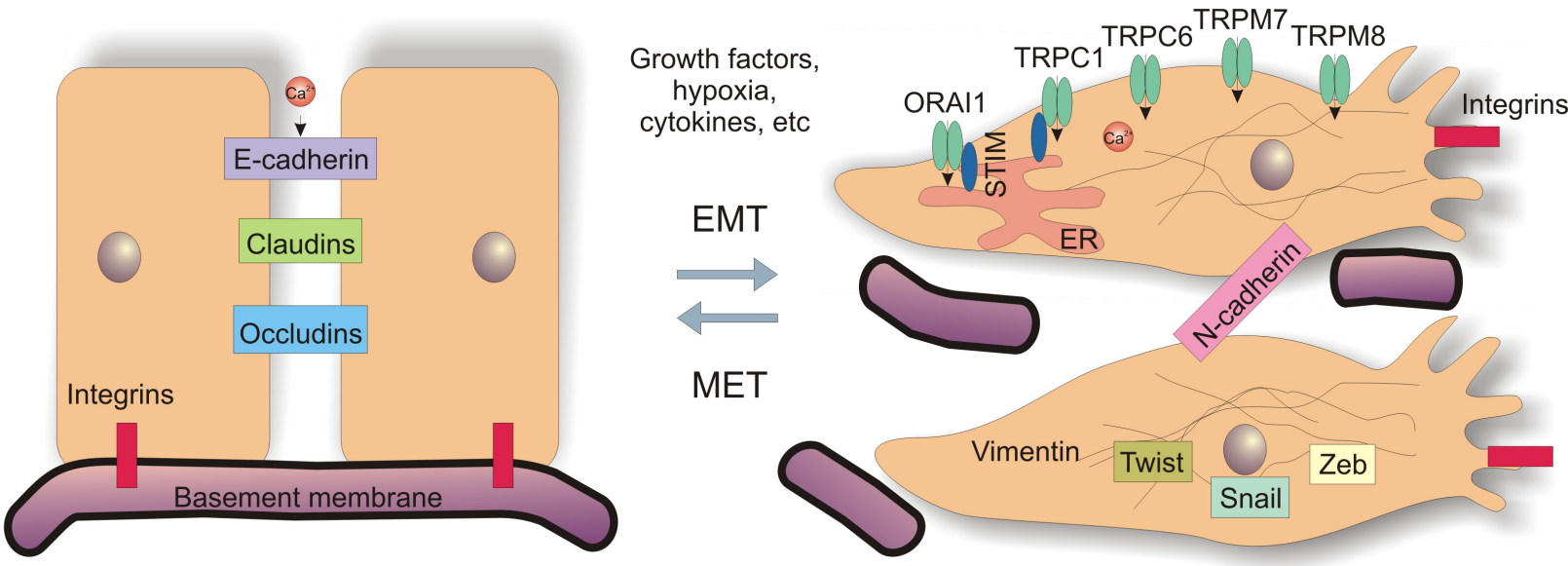

EMT and loss of cell-cell contact
[Ca2+]i peak attenuation and sustained influx after EMT stimulators
Ca2+ signaling remodelling as regulator of EMT markers
Cancer cells
Directed cell migration
Ca2+ distribution according to the front-rear end polarity
Ca2+ influence on cyto-skeleton remodelling,focal adhesion turnover
Healthy epithelial cells
Basement membrane
ECM
Invasion
Ca2+ oscillations induce invadopodia formation
Ca2+ influx promotes proteolytic activity
Local angiogenesis
Ca2+-dependent VEGF release from cancer cells
VEGF and ATP mediated Ca2+ signals provide proangiogenic effects specifically on tumour-derived endothelial cells
Blood vessels


Ca2+
E-cadherin
Claudins
Occludins
Integrins
Basement membrane
Growth factors,
hypoxia,
cytokines, etc
EMT
MET
TRPC1
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TRPM7
TRPM8
ORAI1
STIM
Ca2+
ER
Integrins
N-cadherin
Vimentin
Twist
Snail
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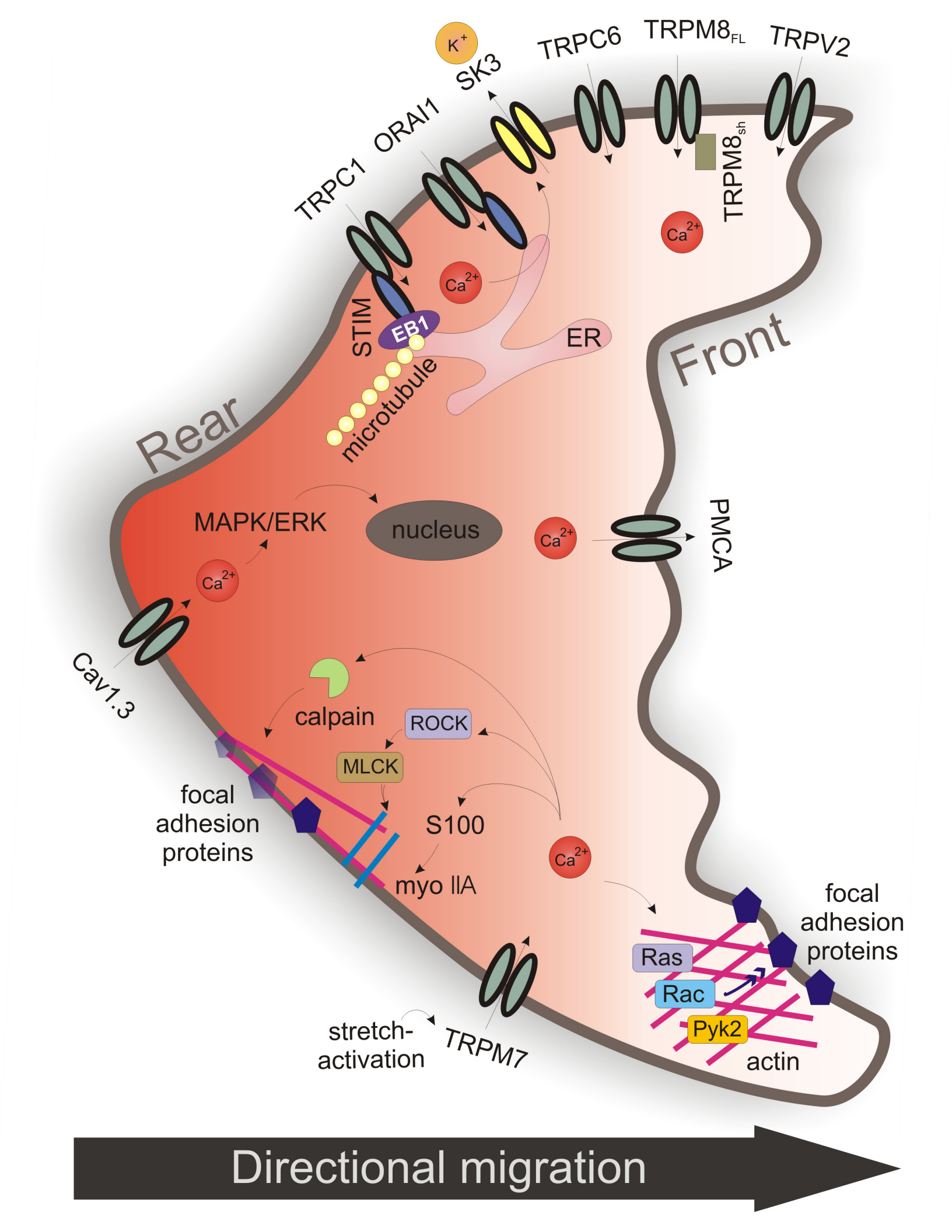
K+
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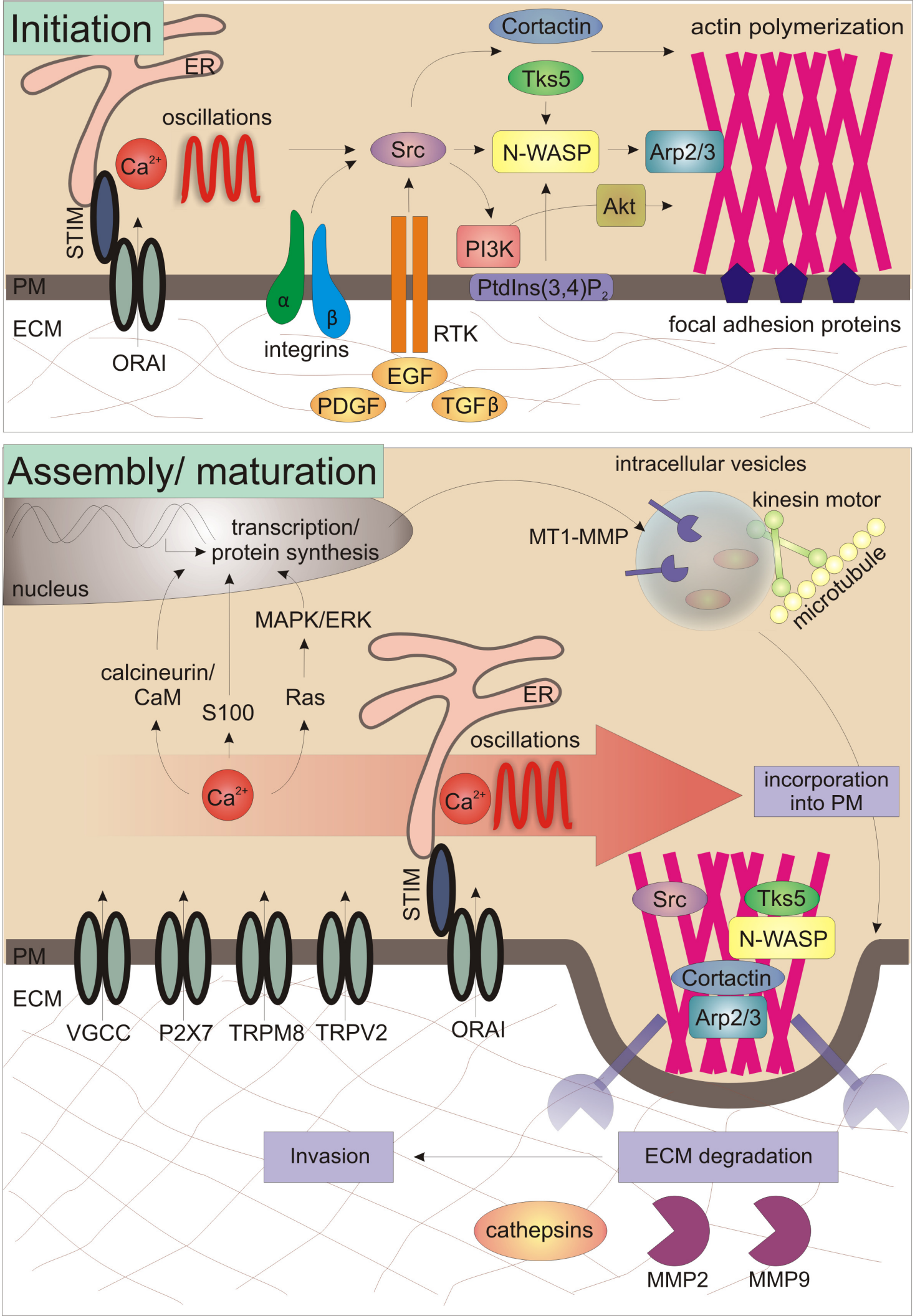
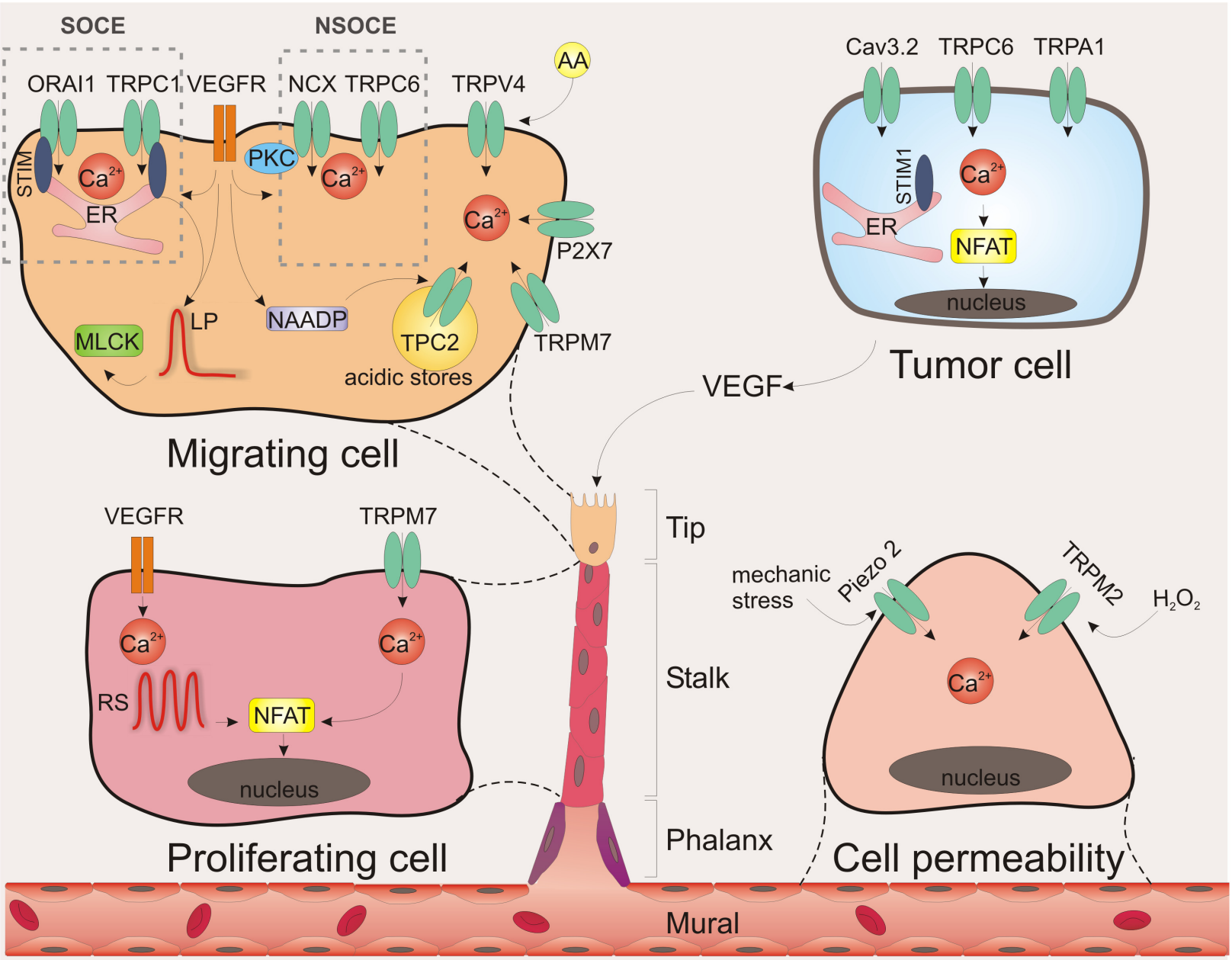
MAPK/ERK
nucleus
PMCA
Cav1.3
calpain
ROCK
MLCK
focal adhesion proteins
S100
myo IIA
Ras
Rac
Pyk2
actin
stretch-activation
TRPM7
Directional migration


Initiation
ER
oscillations
Ca2+
STIM
ORAI
ECM
PM
α
β
integrins
RTK
EGF
PDGF
TGFβ
Src
Cortactin
Tks5
N-WASP
Arp2/3
Akt
PI3K
PtdIns(3,4)P2
actin polymerization
focal adhesion proteins
Assembly/ maturation
intracellular vesicles
kinesin motor
MT1-MMP
microtubule
transcription/
protein synthesis
nucleus
MAPK/ERK
calcineurin/
CaM
S100
Ras
ER
oscillations
Ca2+
Ca2+
incorporation
into PM
STIM
PM
ECM
VGCC
P2X7
TRPM8
TRPV2
ORAI
Src
Tks5
N-WASP
Cortactin
Arp2/3
Invasion
ECM degradation
cathepsins
MMP2
MMP9


SOCE
NSOCE
ORAI1
TRPC1
VEGFR
NCX
TRPC6
TRPV4
AA
STIM
Ca2+
ER
PKC
Ca2+
Ca2+
P2X7
MLCK
LP
NAADP
TPC2
acidic stores
TRPM7
Migrating cell
Cav3.2
TRPC6
TRPA1
STIM1
Ca2+
ER
NFAT
nucleus
Tumor cell
VEGF
VEGFR
TRPM7
Ca2+
Ca2+
RS
NFAT
nucleus
Proliferating cell
Tip
Stalk
Phalanx
mechanic stress
Piezo 2
TRPM2
H2O2
Ca2+
nucleus
Cell permeability
Mural